

# THE MICROBIOLOGY OF INSECTS

## WITH SPECIAL REFERENCE TO THE BIOLOGIC RELATIONSHIPS BETWEEN BACTERIA AND INSECTS

EDWARD A. STEINHAUS

*Department of Bacteriology, Ohio State University, Columbus, Ohio*

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It is noteworthy that one of the first instances of an animal disease shown to be caused by a microorganism was Pasteur's discovery of the etiologic agent of pébrine, a microsporidian disease of silkworms. This dramatic beginning in the study of the relationships between microorganisms and insects does not, however, seem to have maintained itself. The only type of microbiologic-entomologic relationship that has been developed to any great extent is that of the rôle of arthropods as hosts and vectors of the agents of human, animal and plant diseases. Important as this aspect of the subject is, it should not be developed to the exclusion of other important relationships between microorganisms and insects. More intimate associations between the members of these two biologic groups are, with few excep-

tions, little known and appreciated. The problems arising out of these associations are relatively unexplored. Such relationships are of still greater consequence when there exists the possibility of transferring experimental results to the study of arthropod-transmitted diseases of man, animals and plants. Such possibilities have already been demonstrated in the case of the diseases due to viruses, rickettsiae, and other agents. Further investigation of the little-studied symbiotic associations between microbes and insects would greatly increase the knowledge of physiology not only of insects and microbes themselves, but of living things in general. The successful use of microbial agents as a method of insect control likewise rests largely upon a more thorough understanding of the intimate relationships between microorganisms and insects. These and many other important problems could be clarified and better understood if more knowledge existed concerning the microbiology of insects.

This review is not intended to be a compilation of the results of all the investigations made in this field. Instead, it is an attempt to systematize the subject and to assist in securing for it a place in the attention of those interested in microbiology and entomology. It should be emphasized that many of the biologic relationships between microorganisms and insects have, of necessity, not been considered in this review. In general, the discussion has been limited to what may be considered as the flora of normal insects.

If, as has often been said, the principal value of facts is that they give us something to think about, then a gathering and orientation of the main facts of the subject under discussion should be worthy of presentation. To a great extent it will be left to the reader to formulate his own opinions concerning much of the material presented. This necessity is demanded by the lack of sufficient data upon which to form definite conclusions. Nevertheless, certain generalizations are necessary at this stage if there is to be any further progress in the field. This the writer will attempt to do whenever the opportunity presents itself. It is fairly certain that many of the generalizations to be

made, as well as many of those made by other writers, will have to be modified and re-expressed as the field develops.

#### THE EXTRACELLULAR FLORA OF INSECTS

By reason of the ubiquity of microorganisms in nature it is not surprising that the intestinal tract of many insects should be found to contain large numbers of microbes. If one makes a microscopic examination of the contents of the alimentary canal of an insect, he may find all or any one of the chief microbial forms of life: bacteria, yeasts, molds, and protozoa. In most cases the bacteria predominate in numbers over the other forms. Moreover, one or several species of bacteria may be constantly represented in the flora of the digestive tract of any given species of insect.

##### *Flora of the cecal pouches*

Probably the first observer to record the presence of living microorganisms in insects was Raimbert (1869) who obtained anthrax bacilli from flies experimentally exposed to the cadavers of infected animals. Another of the early investigators to observe the presence of bacteria in the intestinal tract of insects was Forbes (1882). On examining crushed specimens of the chinch bug reared in the laboratory, he found the fluids to be "swarming with a species of *Bacterium* not easily distinguishable from *B. termo*."<sup>1</sup> This bacterium was found to be much more numerous in that part of the intestinal canal posterior to the malpighian tubes. Similar results were obtained from observations on chinch bugs taken from the field, although nothing of the sort could be detected in the fluids of the corn plant louse (*Aphis maidis*) which fed on the same stalks.

Later on, Forbes (1892) reported on the bacteria normal to the digestive organs of Hemiptera. He noted that certain appendages to the alimentary canal in the members of the families Scutelleridae, Pentatomidae, and in certain Lygaeidae and Coreidae contained large numbers of bacteria. In the Coreidae

<sup>1</sup> Unless otherwise indicated, all scientific names used in this review, regardless of how they are designated in present-day nomenclature, are those used in the publication cited.

and Lygaeidae these cecal structures were present in one genus and absent in another of the same family. Only the higher Hemiptera (Pentatomidae, Scutelleridae, Corimelaenidae, etc.) invariably possess them. They are always absent in the lower Hemiptera. In the absence of these structures, the microorganisms are not found in any other part. According to Forbes, the gastric pouches of grasshoppers, cockroaches, and carabid beetles do not commonly contain bacteria. In the Hemiptera the gastric ceca are located near the posterior end of the mid-intestine, while in many other insects, particularly the Orthoptera, Coleoptera, and Diptera, the ceca occur near the anterior end. Incidentally, in the light of a relationship to be discussed later, it is interesting to note that Forbes mentioned the fact that the fat bodies of various cockroaches contained structures that appeared to be bacteria. With respect to the chinch bug, this investigator stated that the organism most commonly present in the intestinal tract was one he called *Micrococcus insectorum*.

Continuing these studies, Glasgow (1914) described in greater detail the bacteria-containing ceca of the Heteroptera (Hemiptera) and revealed many interesting facts. The ceca of a given species of insect, regardless of the geographic source of the specimen, are invariably filled with a pure culture of a single species of bacterium. Morphologically, the bacteria from different hosts vary greatly, ranging from minute, coccus-like bacilli to huge spirochete-like forms; but in whatever insect they occur, they are morphologically characteristic for the particular species harboring them. Glasgow believed that these strikingly different forms of bacteria really belong to a single, clearly defined group and that the differences in structure are due to the effect of some specific physiologic peculiarity of the insect. It would be interesting to know what credence modern students of bacterial dissociation and variation would give this interpretation. (See also Paillot, 1931, 1932.) That these organisms are bacteria was indicated by culture experiments and agglutination tests. Most of the bacteria from the various species of insects could not be grown by Glasgow on ordinary culture media, although those from *Anasa tristis* were cultured in nutrient broth.

These bacteria, normally present, appear not only to inhibit the development of foreign bacteria, but to exclude them altogether. The mid-intestine is usually wholly free of the invading bacteria and protozoa commonly present in many related insects. According to Glasgow, this is probably the chief function performed by the cecal bacteria in the life processes of the host. Since the ceca of the Hemiptera appear to contain definite species of bacteria, the phylogenetic significance and function of the ceca are of interest. There are complete gradations from extremely simple to very complex forms. Glasgow concluded that one function of these appendages is merely to provide a safe place for the multiplication of normal bacteria. It would seem, however, that their presence is probably more intimately concerned with the life processes of the insect than merely that of serving teleologically as a haven for bacteria.

One of the most interesting features of this bacterium-insect association is that it is congenital, the organisms appearing early in the alimentary canal of the developing embryo. This fact also indicates that a close relationship with the insect exists, although convincing evidence of true symbiosis is lacking. Much work must still be done to determine the true rôle that the bacteria play in the life of the insect. Kuskop (1924) also deals with this in a paper containing a tabular survey of these bacteria-containing ceca.

#### *Flora of the digestive tract proper*

Although the novelty of the cecal bacteria induces one to give them first attention, the microbial flora of the digestive tract itself is no less important and interesting. It was realized early by those studying diseases of insects that before conclusions could be made concerning the abnormal flora of the insect, the normal flora would have to be studied. This was exemplified by the work of White (1906) in his studies on the diseases of bees. In this investigation he also studied the bacteria of the normal combs, pollen, honey, larvae, adults, and the intestines of healthy bees. Both on the surfaces of the adult bees and on the combs there occurred quite constantly a species of bacterium referred to as "Bacillus A," which White believed to be the organism

confused by some workers with *Bacillus alvei*, the cause of European foul brood. In the pollen and in the intestines of the adult bees a species referred to as "Bacillus B" occurred very constantly. From the combs, *Bacterium cyaneus*, *Saccharomyces roseus*, and a micrococcus were isolated and studied. The normal larvae were, as a rule, sterile, as was the honey from a healthy hive. It is interesting to note that White quite constantly found an anaerobe in the intestine of the healthy bee. In addition, he isolated from the intestine the following microorganisms: *Bacillus cloacae*, *B. coli communis*, *B. cholerae-suis*, *B. subgastricus*, *Bacterium mycoides*, *Pseudomonas fluorescens liquefaciens*, and two forms referred to as "Bacillus E" and "Saccharomyces F."

During the years following 1906, the bacterial flora of the housefly (*Musca domestica* L.) as well as that of several species of cockroaches aroused considerable interest (Longfellow, 1913; Barber, 1914). Jackson (1907) found as many as 100,000 human fecal bacteria in a single fly, and recognized that these bacteria might easily survive passage through the intestinal canal of the insect. Graham-Smith (1909) examined 148 flies caught in various parts of London and Cambridge. Of these, 35 (24%) possessed externally or internally, or both, bacilli belonging to the colon group. Later (1913), he reported that *B. prodigiosus* could be cultivated from the contents of the crop and intestine of the housefly in large numbers up to 4 or 5 days after inoculation, and was found surviving in the intestine up to 18 days. Graham-Smith also states that although it seems to have been proved that the spores of *B. anthracis* may survive after being ingested by fly larvae, most observers agree that such non-spore-bearing pathogenic organisms as *B. typhosus*, *B. enteritidis*, and *B. dysenteriae* derived from cultures and added to the food of the larvae are not present in the flies which emerge, except under very special and highly artificial conditions. Somewhat earlier, however, Bacot (1911) reported that when the food of some newly hatched larvae of *Musca domestica* was inoculated with a culture of *Bacillus pyocyaneus*, viable bacteria remained in the gut during metamorphosis.

Torrey (1912) found that flies examined up to the latter part

of June were free from fecal bacteria of human origin and carried a homogeneous flora of coccal forms. During July and August, periods occurred during which the flies examined possessed several millions of bacteria, alternating with periods in which the number of bacteria was reduced to hundreds. Bacteria of the colon type were first encountered in abundance during the early part of July. The bacteria in the intestines of the fly were 8.6 times as numerous as those occurring on the external surface of the insect. Another example of seasonal incidence has been observed in the case of the bacteria producing soft rot of potatoes. In this case, the bacteria pass the winter in the digestive tract of the puparia of *Hylemyia cilicrura* Rond. (Leach, 1933). Nicoll (1911), and Cox, Lewis, and Glynn (1912) also studied the numbers and varieties of bacteria associated with the housefly, finding large numbers of the coliform type. (See also Hewitt, 1914.)

Bacot (1914) made similar studies on the bacteria of the alimentary canal of fleas during their metamorphosis, and found that the alimentary canal of the flea larva may become "infected" with the following bacteria if they are mixed with its food: *B. pyocyaneus*, *B. enteritidis*, *Staphylococcus aureus*, and *S. albus*. He also showed that an infection of the larval gut may persist until the resting period of the larva in the cocoon, and that there is no satisfactory evidence that such an infection can survive the pupal stage.

From this example, it can be seen that a knowledge of the fate of the microbial flora of an insect during metamorphosis is important from a practical standpoint; yet almost nothing is known about this matter. Bacot (1911) recognized the importance of the persistence of a bacterial "infection" in the larval gut of an insect through the period of metamorphosis and its survival in the adult after emergence from the pupa. This fact is especially important from a public health standpoint. If, for example, the larva of the housefly becomes a reservoir for organisms pathogenic to man and then transmits them when it is an adult, the chances of spreading disease are much greater. Furthermore, in deciding what is the normal flora of an adult insect,

one must consider adventitious organisms that have been picked up not only by the imago itself, but by the larva as well.

The bacterial flora may vary in different parts of the alimentary tract. For example, the milkweed bug, *Oncopeltus fasciatus* Say, has a distinctly different bacterial flora in its pylorum and rectum from that in the four stomachs which precede them (Steinhaus, 1940). The predominant bacterium isolated from the pylorum and rectum is one closely associated with the *Proteus* group of bacteria, while in the four stomachs the main flora consists of a species of the genus *Proteus* which differs from that found in the pylorum and rectum, and an organism very similar to those of the genus *Eberthella*.

*Numbers of organisms with respect to location in insect*

The numbers of organisms may also vary with the region in which they are located. The digestive tracts of some insects have been found to be sterile while others are packed with organisms. Hertig (1923) points out that in the honey bee the greater number of organisms is found in the hind intestine, particularly in the rectum, while relatively few both in numbers and variety are found in the ventriculus, except at times of food accumulation. In fact, this worker states that at times he obtained no growth at all in the media inoculated with a small section of the wall and contents of the ventriculus. He explains that this slight bacterial content of the ventriculus is due, perhaps, to the fact that solid particles pass rapidly to the hind-intestine, and further, that the contents of the ventriculus are at times rather acid, which may inhibit the multiplication of organisms. Stammer (1929) in a study of 37 species of trypetids showed the presence of bacteria in all cases. Their manner of distribution varied with the genus of the host. In the simplest case the bacteria were diffuse or in clumps in the intestinal contents of the larva and young adults. In old adults they were always present in enormous numbers in the lumen of the intestine. In the larvae of Tephritini, Schistopterini, and *Dacus oleae*, the diverticulum of the esophagus contains the bacteria. Melampy and MacLeod (1938) state that in the case of *Agriotes mancus* Say, the greatest number of bacteria was



found in the hind-gut. Thorpe (1930) reported a similar condition in the petroleum fly (*Psilopa petrolii*).

It is definitely known that the digestive tract of some insects, such as certain members of the blood-sucking group, is sterile. In some cases only certain parts of the tract are devoid of microorganisms. An example of this regional sterility is represented by blow-fly maggots used in the treatment of slow-healing wounds such as in osteomyelitis. Using the larvae of the blow-fly *Lucilia sericata*, Robinson and Norwood (1933, 1934) found that large numbers of the bacteria taken in with the food were destroyed in passing through the long, tubular stomach of the maggot. No viable bacteria were found in any cultures of the intestine. However, in all of the specimens dissected, abundant bacterial growth was obtained from the fore-stomach. The intermediate area, the hind-stomach, showed slight growth of microorganisms in one-third of the cases. (See also Simmons, 1935.) Duncan (1926) found that the feces of certain other insects were sterile. This worker also studied the nature of the bactericidal properties of insect feces. Nuttall (Herms, 1939) found that the anthrax bacillus died in the stomach of the bed bug in 48 to 96 hours at 13° to 17°C. and in 24 to 28 hours at 37°C., although the feces from the bugs contained living bacilli during the first 24 hours after feeding. Chapman (1924) examined the digestive tract of the confused flour beetle, *Tribolium confusum* Duv., and failed to find any living organisms present.

#### *The nature and kinds of bacterial flora*

As to the nature and kinds of bacteria comprising the flora of insects, not very much evidence exists. Very few investigators have attempted to identify or classify properly the bacteria they have isolated. For this reason we frequently see an organism referred to simply as a bacillus, coccus, bacterium, or coccobacillus. Because of these indefinite and ambiguous terms the true nature of the bacterial flora is not clearly defined. Just what types and groups of bacteria predominate in insects is difficult to say with certainty. A recent survey of the bacterial flora of certain insects (Steinhaus, 1940) indicates that most of the major

types of bacteria are represented. These include gram positive and negative short rods, gram positive spore-forming bacilli, and gram positive cocci. The gram negative short rods predominate, comprising slightly more than 50 per cent of the bacterial flora of the intestinal tracts of the insects studied.

Among other things, the reviewer's survey revealed a number of bacteria which were elliptical in shape and which one is prompted to designate as coccobacilli. They varied in their physiologic characteristics as well as in their reaction to the gram stain. These forms, however, may be pleomorphic types of the familiar short rods and cocci. The writer is not willing to go as far as have some workers, such as Pospelov (1926), who states that each species of insect has its own species of coccobacillus. Certainly, experimental evidence does not warrant such a sweeping statement. Nevertheless, it does appear that the presence of the coccobacillus, as a morphologic type, is characteristic of insects and this form may merit a taxonomic grouping of its own after a more thorough investigation of the subject. It is evident that steps should be taken to make clear just what is meant by the term "coccobacillus." Glaser (1918), in undertaking a systematic study of a number of cultures which were designated as *Coccobacillus acridiorum* d'Herelle, found that some of the separate cultures proved to be either different species or varieties of the same species. "This fact," says Glaser, "may account for some of the contradictory views held by so many workers and it is my hope that this article will also demonstrate the need for attention to the ordinary principles of bacteriology which seem to be so persistently neglected by many entomologists." (See Paillot, 1913; DuPorte and Vanderleck, 1917.)

Many peculiar forms are found in the bacterial flora of insects. During an investigation by Roberts (1935) of the intestinal flora of several termites from central Texas, a peculiar bacterial species was observed as a normal inhabitant of the termite intestine. The outstanding characteristic of the organism, which he named *Bacillus rotans*, is the mobility of young colonies on the surface of nutrient agar. (See also Smith and Clark, 1938; Clark, 1939.)

*Non-bacterial flora of insects*

Besides bacteria, other microorganisms are frequently found associated with insects. Rickettsiae, yeasts, molds, as well as protozoa have all been found living freely in the intestinal tract of arthropods. Jungmann (1918) noticed that *Rickettsia melophagi* was present in the stomach lumen and on the surface of the stomach epithelium of mature individuals of the sheep louse, *Melophagus ovinus*, but only rarely in the young individuals. The higher fungi, particularly the molds, have frequently been isolated from insects. Schaudinn (1904) was perhaps the first to describe a fungus as a significant normal inhabitant of an insect. In the mid-intestine of several species of Culicidae he found a fungus which he believed produced an enzyme that passed into the wound during the act of sucking and not only prevented coagulation of blood but also caused subsequent irritation and swelling.

With insects, as with other animals, the main association of viruses has been in connection with insect, plant and human diseases. Perhaps with the increase of our knowledge of viruses themselves and the perfection of techniques in the study and detection of non-pathogenic viruses, it will be found that insects harbor even these agents as normal inhabitants. It is noteworthy that insects have been found to harbor bacteriophage (Glaser 1938).

## THE RÔLE OF MICROÖRGANISMS IN THE NUTRITION OF INSECTS

Since microorganisms maintain such an intimate relationship with insects and since such a large number is harbored in the intestinal tract, one of the first probable effects of this relationship is that of the influence of microorganisms on insect physiology and nutrition.

*Bacteria as a source of food*

Bacteria may not only be related to the food habits of an insect, but they may also serve as food itself. The literature on this phase of the subject is both scattered and contradictory. It will be our purpose here merely to indicate the nature of the work

done and thus to reveal the fertility of the field for further research.

Mitchell (1907) early expressed the belief that the "wiggler" of *Stegomyia fasciata* is preëminently a bacteria-feeder, because the larvae develop rapidly in water contaminated with sewage. In later years her belief was supported by the work of Bacot (1916), Atkin and Bacot (1917), Barber (1928), Rozeboom (1935) and others. That mosquito larvae may live in the absence of bacteria has been shown by Trager (1935a, 1935b). In Bacot's report the suggestion that the bacteria themselves served as food for the mosquito larvae was based on the clearing action the latter displayed in water, originally turbid from its enormous bacterial content, in conjunction with the fact that the gut-contents of larvae taken from this water showed relatively few bacteria. He attributed the scarcity of bacteria to their being rapidly digested. Barber found that a combination of bacteria with infusoria or with algae seemed to afford the best conditions for the growth of *Culex quinquefasciatus* and of *Aedes aegypti*. No considerable growth of larvae was obtained in sterile nutrient media nor in cultures of the insect provided only with dead organic material. Howland (1930) observed that algae were ingested by many species of mosquito larvae and appeared to form an important part of their food. (See also Senior-White, 1928; Hinman, 1933.) A relationship similar to that of the mosquito larvae in contaminated water was suggested by von Wolzogen Kühr (1932) with the larvae of *Chironomus plumosus* which frequented sand-filters in the summer. This was attributed to the presence in the filters of *Pseudomonas fermentans* upon which the larvae supposedly fed. A similar situation was described by Dyson and Lloyd (1933) in sewage beds.

Although most workers assume that the microorganisms ingested by mosquitoes actually serve as food, one must be careful to distinguish this from the fortuitous ingestion of large numbers of organisms that play no important part in the nutrition of the insects. Such is the case with various species of Geotrypes which Vaternahm (1924) found did not contain an indigenous bacterial flora but only that received with the food (dung).

One of the first to advance the idea that bacteria are indispensable to growth of certain insects was Bogdanow (1906), who found that the larvae of *Calliphora vomitoria* fail to develop in the absence of microorganisms. Later (1908), Bogdanow stated that the larvae require a definite and fairly simple bacterial flora. Sterile larvae on sterile food never developed normally, although some of them reached the pupal stage. Weinland (1907), however, showed that the larvae of *Calliphora* are able to digest meat without the assistance of bacteria. Bogdanow also found that larvae of the housefly, *Musca domestica*, can be bred on starch paste or gelatin, but only in the presence of molds and bacteria. Wollman (1921) reported, however, that microbe-free cultures of flies can be maintained indefinitely, as can also similar cultures of the moth *Galleria melonella*. The work of Glaser (1924) showed that the growing larvae of flies were dependent on certain accessory growth factors which may be obtained from bacteria and yeasts, but that microorganisms and their activities are not absolutely essential to the normal growth, development and longevity of the flies. Later (1938) he developed a method whereby houseflies may be raised in sterile culture, free from microorganisms. Baumberger (1919) reported that the larvae of the fly *Desmometopam-nigrum* Zett. are probably always dependent on microorganisms and that the larvae of the housefly very probably feed on microorganisms. Trypetidae larvae can develop only when microorganisms are present, according to Stammer (1929).

#### *Yeasts and molds as food for insects*

Although bacteria alone may serve as food, considerable work has been done on the utilization of yeasts and molds as food, either one alone or together with bacteria.

In 1913 Guyénot reported, in one of a series of notes, that bacteria-free larvae of *Drosophila ampelophila* Loew. may breed entirely on yeast. Under natural conditions the larvae feed principally on yeast and other microorganisms; and the absence of microorganisms renders certain foods unsuitable. (See also Guyénot, 1917.) Northrop (1917) observed that the number of flies may be increased by the addition of banana, casein, or sugar

to the yeast. Loeb and Northrop (1917) went a step farther and showed that while the larvae of *Drosophila* cannot grow on glucose agar unless yeast is added, the imago can live well on glucose agar alone. Baumberger (1917) maintained that the insect depends on yeast for its protein.

Later, in a very thorough report on a nutritional study of insects with special reference to microorganisms and their substrates, Baumberger (1919) clarified the situation with respect to *Drosophila melanogaster*. Sterile larvae grow rapidly on sterile food but die before pupating. Decaying fruit is not the food for *Drosophila* but merely a substrate for yeast cells, although the fruit also has some additional nutritive value. Further, the larvae grow on dead as well as on living yeast. Other microorganisms (bacteria and molds) are also suitable food, but yeast is a more complete food. In general, the use of microorganisms as food is widespread among insects. According to Baumberger, the feeding habits of insects may be grouped into three classes, as follows:

1. Ingestion of microorganisms with substrate, e.g., *Drosophila*, *Musca*, *Sciara*, worker termites.
2. Feeding directly on microorganisms, e.g., tree crickets, many adult Diptera.
3. Preparation, by insects, of a substrate for the development of microorganisms, e.g., leafcutting ants, termites, ambrosia beetles.

As indicated by the third class of feeding habits, the higher fungi present many interesting relationships with insects. One of the early observed types is exhibited by the fungus-growing termites and ambrosia beetles. The carefully nurtured and "cultivated" fungus apparently furnishes these insects an ample supply of food. This relationship has been well described by Hingston (1929) who, in speaking of the ant *Atta sexdens* of Brazil, states: "The ants . . . ascend the foliage, cut pieces from the leaves and carry them back to their nest. . . . They cut them up into fragments, mould the fragments into sponge-like masses which will serve them as underground gardens. On these gardens they grow a fungus, a small white mushroom-like type of vegeta-

tion, on which the ants themselves feed and which they supply to the young ants in the nest. Each species of leaf-cutting ant grows its own particular species of fungus, and none but this particular species of fungus is allowed to grow in the nest." This author goes further to describe just how these gardens are planted and fertilized by the ants.

According to Buchner (1928), the fragments of wood bitten off by bark beetles undergo digestion in the insects' digestive tract. However, the wood is first transformed from a poor substrate into a rich food by a fungus. Each species cultivates a specific fungus. There is a dense outgrowth of fungus on the walls of the burrows of the bark beetles, and in the case of one (*Sirex*) there are special organs which harbor the fungi. Another type of symbiotic feeding on wood and similar substances is presented by the beetle *Anobium paniceum*, which has special appendages of the mid-intestine containing *Saccharomycetes* in their cells (Escherich, 1900; Buchner, 1921, 1928; Heitz, 1927).

Besides participating in the actual breaking down of the food in the digestive tract, bacteria may exert other influences on the digestive processes. As pointed out by Wigglesworth (1927) even such a factor as the acidity commonly observable in the crop of the cockroach is not a physiologic constant but is dependent on bacterial action. Perhaps more important, however, is the ability of microorganisms to synthesize certain accessory substances which aid in the metabolism of the insect. In many cases such possibilities exist but more experimentation is needed to clarify the matter. For instance, Zabinski (1928) observed that *Blattella germanica* synthesizes tryptophane, but was unable to decide whether this is or is not produced by the activities of symbiotic microorganisms.

Portier (1919) was one of the first to suggest that the source of vitamins for the individual insect is the intracellular organisms it possesses. Wollman (1926) probably overlooked this possibility when he claimed that cockroaches (*Blattella germanica*) may dispense with vitamins. Hobson (1933) supports Portier's hypothesis with his work on the nutrition of blow-fly larvae. He found that these larvae were unable to develop aseptically on

sterile blood owing to the lack of growth factors of the vitamin B type. The presence of bacteria improved growth, and yeast autolysate allowed the larvae to grow at a normal rate. Later on (1935), he reported that the natural flora must supply the necessary vitamins and that larvae grow readily on blood inoculated with pure cultures of various bacilli isolated from the intestine and from blown meat. *Bact. coli* proved equally effective in these experiments. Observations of Wigglesworth (1936) on *Rhodnius prolixus* Stal. support the view that symbiotic organisms in exclusively blood-sucking insects provide an endogenous source of vitamins. (See also Koch, 1933; Trager and Subbarow, 1938.)

Though not of a strictly nutritional nature, a phenomenon first reported by Atkin and Bacot (1917) and Bacot (1917) should be mentioned here. These workers found that in experiments on mosquito eggs (*Stegomyia fasciata*), the greatest stimulus to hatching is the introduction, into their environment, of living yeasts or bacteria. The stimulus produced by killed cultures of bacteria and sterile watery extract of brewer's yeast was more feeble, many of the resistant eggs failing to hatch. These, when treated with living cultures of bacteria such as *Bact. coli*, never failed to hatch. Sterile filtrates of bacteria were less effective than killed cultures. The methods of experimentation were very simple. Different species of living bacteria were introduced into tubes of sterile media, such as peptone water, in which the eggs had been lying dormant for 11 to 15, and in some cases 39 days. Upon inoculation with the bacteria all eggs hatched within 18 hours. Atkin and Bacot explain this phenomenon by supposing that the stimulus is of the nature of a "scent" which penetrates to the larvae lying dormant within the egg shells, causing them to make vigorous movements which result in the uncapping of the egg. Some of the differences of opinion concerning this phenomenon were reconciled by Rozeboom (1934), who found that a great deal depends on the age and condition of the egg. Of 240 old, dry eggs only four hatched in sterile media, whereas 204 hatched within the two days following inoculation of the media. Of fresh, moist eggs, 35 per cent hatched



in distilled water, 51 per cent in sterile, filtered breeding water, and 82 per cent in water contaminated with bacteria. (See also Hinman, 1930, 1932.)

It seems that the ingestion of bacteria and yeasts by insects is of sanitary significance for man, and also may be important for maintaining the insect species. This is emphasized when we remember that the larvae of mosquitoes feed greedily on microorganisms. It is such biologic relationships as these that serve as an incentive for a more thorough experimental penetration of this field.

From the above discussion, we may conclude that insect larvae can be reared on sterile media if they are supplied with all the necessary food factors. As stated by Wigglesworth (1939, p. 286), "If these are deficient, infection with microorganisms (in the case of *Drosophila*, particularly the introduction of yeasts) improves the rate of growth. Sterile *Lucilia* larvae will grow on beef muscle; they fail to grow on guinea pig muscle; but if this is infected with *Bacillus coli* or if a yeast extract is added to it, normal growth takes place. . . . In these cases there is little doubt that the microorganisms are synthesizing the necessary vitamins of the 'B' group."

#### *Microorganisms and the physiology of insect digestion*

Considerable evidence has been advanced that microorganisms may play a greater rôle in the nutrition of insects than merely serving as food. The physiologist is far from knowing just how great a part microorganisms play in the digestion of food materials in the digestive tract. A few interesting examples showing the importance of microorganisms in this respect stand out.

The best known examples are the intestinal flagellates which take an active part in the digestion of cellulose in the gut of the termite (Cleveland, 1924 to 1928), and in the wood-feeding roach *Cryptocercus* (Cleveland, 1924). If, for instance, as Hegner (1938) relates, the termite, *Termopsis augusticollis*, which possesses four different types of flagellates, is kept in pure oxygen for 72 hours at atmospheric pressure, all the protozoa are destroyed but the termite is unharmed. When returned to normal

conditions the termite will eat wood but is unable to digest it and starves. If reinoculated with the flagellates, the termite can again digest the wood and live its normal life. There are other microorganisms associated with the flagellates of termites (Cleveland, 1928; Kirby, 1937), but whether they influence the symbiotic relationship in any way is not clear. Some workers have differed in their interpretation of this phenomenon in wood-eating insects. It has been claimed (Pierantoni, 1936) that the protozoa themselves contain the bacteria which in turn break down the cellulose. Mansour and Mansour-Bek (1934) state that wood-eating insects with organisms living in the intestinal lumen (as in termites and Lamellicorn larvae) use the organisms directly as food and derive no digestive help from them. These authors do not accept the view that such insects live on the products of cellulose, split by the microorganisms they harbor.

Besides protozoa, yeasts and molds are also thought to influence the digestive processes of insects, producing enzymes which are ordinarily lacking in the digestive system. On the other hand, investigators have found that the higher fungi play no rôle in the process of digestion in many insects. Brown (1928) has pointed out that extreme care must be exercised in work on the digestive enzymes of insects to exclude those produced by microorganisms, especially by bacteria. Mahdihassan (1935), in his studies on the "symbiotes" of scale insects, suggests that the presence of yeast-like forms of "symbiotes," as opposed to bacterial forms, is physiologically associated with the excessive formation of sugars. Such insects may either excrete the sugars as honey dew or further transform them into waxes or into lac.

Petri (1905) was one of the earliest to assign to the bacteria a definite digestive rôle. The bacteria constantly present in the gastric ceca of the olive fruit fly (*Dacus oleae*) were found to produce lipase. It is suggested that the activity of the bacteria in the digestion of fats must be very important for the larva which feeds on the olive, a fruit rich in fats. In a later paper (1910), he asserts that partial digestion of the oil might be possible without the aid of bacteria, since many larvae living on seeds rich in oil do not possess intestinal bacteria. Bogdanow

(1906) believed that the formation of ammonia during larval development of *Calliphora vomitoria* is not a characteristic of protein digestion by the larvae but probably a result of bacterial activity. Weinland (1907), on the other hand, insisted that the ammonia is the result of larval metabolism. Wollman (1911, 1921) indicates that Weinland was mistaken; no ammonia is produced by sterile larvae and its production is due to microorganisms. (See also Hobson, 1932.) Weinland (1908) observed further that bacteria take no part in the process of fat formation in the larvae. Guyénot (1906, 1907) found that muscid larvae (mostly those of *Lucilia*) are unable to produce any digestive ferments which liquefy meat. He believed that this is accomplished by bacteria. In contradistinction, Wollman (1921) claims that aseptically bred larvae liquefy gelatin, which indicates that they produce some proteolytic ferments.

Portier (1911) claims that leaf-mining larvae of *Nepticula malella* and *Gracilaria syringella* live under sterile conditions and do not harbor any microorganisms in their bodies. On the other hand, the normal leaf-feeding larva of the silkworm, *Bombyx mori*, has its digestive tube populated with microorganisms, some of which destroy the wall of the leaf cell, while others thrive on its contents. The larva uses the cell contents directly as food. Glaser (1925), however, reared large numbers of silkworms and rarely found many bacteria in the digestive system of normal worms. Where bacteria became numerically high, the worms ailed and died. Hering (1926) criticized some of the views of Portier, stating that up to that time no true "symbionts" were known in leaf-miners. Werner (1926) found that the digestive tract of the larva of *Potosia cuprea* Farb. has a very rich microflora able to cause the fermentation of cellulose. A specific bacterium was isolated and named *Bacillus cellulosam fermentans*. Schütte (1921) found that cellulose is digested by the larva of *Hydromyza livens* F., but apparently without the aid of bacteria.

It should be remembered, when one is considering the rôle of cellulose-fermenting bacteria in the nutrition of insects, that in most phytophagous insects the food passes through the gut so rapidly (Wigglesworth, 1939, p. 271) that no great amount of

fermentation is likely to take place. The breakdown of cellulose by bacteria is usually too slow a process to be initiated and completed in the few hours during which food remains in the gut. On the other hand, cellulose-splitting bacteria are often associated with the food ingested by insects and for this reason cannot be completely ignored. Furthermore, certain insects, such as the Lamellicorn larva, possess a "fermentation chamber" which is probably used for such purposes (Mingazzini, 1889a, b). Ripper (1930) points out that in the larvae of *Dorcus* and *Osmoderma*, there is no evidence of cellulose breakdown even though the fermentation chamber is full of bacteria.

In 1919 Roubaud asserted that adult tsetse flies were exclusively hemophagous. The blood ingested by the flies was digested only in the middle section of the intestine where the epithelial cells include symbiotic organisms. According to Roubaud, these organisms play an important part in the digestion of blood. Wigglesworth (1929) states, however, that there is no evidence that these organisms play any part in the digestion of the blood.

The true function of the bacteria living in the cecal pouches of various Hemiptera is not altogether clear. Glasgow (1914) and Kuskop (1924) both believe that the bacteria contained in the cecal pouches exclude foreign bacteria altogether. Elson (1937) believes that, since bacteria are absent in the predatory and semi-predatory species of Hemiptera, the presence or absence of microorganisms bears an important relationship to the food habits of the insect. Wigglesworth (1939) states that there is no evidence that bacteria in the ceca of the Hemiptera play any part in digestion.

#### INTRACELLULAR FLORA OF INSECTS

Perhaps the most interesting of all the biologic relationships between microorganisms and insects are those of intracellular parasitism and symbiosis. Within the tissue cells of many arthropods may be found apparently living, non-pathogenic organisms. In some cases, such as the rickettsiae pathogenic for

lice, the intracellular organisms may exert a deleterious influence on the host, but usually they are not only harmless but may be distinctly symbiotic. For this reason such microorganisms have been termed "symbionts" or "symbiotes"<sup>2</sup> by some writers with the assumption that they live in a symbiotic relationship with the arthropod host. This has been the viewpoint from which Buchner (1921b, 1930) has ably reviewed the field. Other excellent reviews of the subject have been presented by Glaser (1930c) and Paillot (1933), and it is unnecessary to repeat their detailed accounts here. Only the high points of the subject will be discussed to indicate the very intimate biologic relationships between microorganisms and insects. It should be mentioned that similar associations exist between microorganisms and arachnids. (See Mudrow, 1932; Gregson, 1938; Trager, 1939.)

Among the earliest to recognize the presence of intracellular "organisms" in the normal eggs and body tissues of insects was Blochmann (1886). He noted that bacteria-like bodies were present both in the eggs and follicular membranes of ants and wasps, and suspected that these forms were bacteria. In the following two years Blochmann (1887, 1888) indicated more confidence as to the nature of these bodies, which were also found in the fat tissue and eggs of *Periplaneta orientalis* and *Blatta germanica*. His contention that they were bacteria was based on their reaction to various reagents and stains, their multiplication by fission, and their method of infection through the eggs. His attempts to cultivate the "organisms" in beef-peptone-gelatin-agar media were unsuccessful. During the next few years Wheeler (1889), Cholodkowsky (1891), and Heymons (1895) confirmed these observations.

The aphids were among the first insects studied with respect to their intracellular "organisms." As early as 1850 Leydig

<sup>2</sup> Both of these terms are used interchangeably throughout the literature. Although "symbiont" is considered by some writers to be a misnomer, it is used more frequently perhaps than the term "symbiote," from the Greek word for "partner" or "companion."

observed certain organs in aphids which have subsequently been called "symbiotic organs," "pseudovitellus," "green body," and "mycetome" or "mycetom." The cells making up these organs are usually known as "mycetocytes" or "bacteriocytes" and it is in these that the intracellular forms are usually located. Following Leydig's report, the nature, origin and development of these organs were described by many workers including Huxley (1858), Balbiani (1866 to 1871), and Tannreuther (1907).

Krassilstchik (1889, 1890), however, seems to have been the first to begin to shift the emphasis from the anatomic study of the mycetome to a study of the bacteria-like forms within this organ. He named these forms "biophytic bacteria." Pierantoni (1909 to 1911) and Šulc (1910) attempted to show that the forms within the mycetocytes were living organisms related to the yeasts. It was Šulc who suggested the name "mycetom" for the "symbiotic" organ, and "mycetocyte" for the individual "symbiotic" cell. (See also Klevenhusen, 1927.)

Not only aphids but many other insects have been reported to possess mycetomes and intracellular organisms. Buchner (1912, 1921) has published excellent accounts of what he considers symbiosis in aleyrodids, coccids, aphids, psyllids, cicadellids, blattids, Cicada, Hymenoptera, Coleoptera, and Lepidoptera. An account of the intracellular inclusions and the embryologic development of the mycetome in aphids has been presented by Uichanco (1924). Mansour (1934a, b) has furnished considerable data concerning the intracellular microorganisms in coleopterous insects; and Gier (1936) has studied the structure and behavior of the intracellular "bacteroids" of roaches. (See also Blochmann, 1892; Florence, 1924.)

Interesting light-producing organs and groups of cells, comparable to the mycetomes and bacteriocytes, have been found in some insects as well as in certain other animals. According to some writers (Buchner, 1921b; Wallin, 1927) these organs are associated with luminescence in certain insects and harbor "phosphorescent" or luminiferous bacteria. These authors also cite various claims which have been made concerning the artificial cultivation of these luminous bacteria.

*Transmission of intracellular microorganisms*

One of the interesting aspects of this study is the nature of the transmission of these organisms from parent to offspring. Kras-silstchik, in his reports on the aphid, observed not only that they are transmitted directly from one generation to the next, but also that they are present in every representative of the species, including the embryo. Uichanco (1924) traced the path of transmission of the symbionts from the follicular epithelium of the parent to the definitely formed mycetome in the offspring. Mansour (1934a), working with certain Bostrychid beetles, reported the transmission of microorganisms from one generation of the host to the next thus: The microorganisms from the mycetomes invade the lobes of the testes, multiply, and mix with the sperm. The microorganisms then pass with the sperm during copulation into the bursa copulatrix of the female. From this region they pass through the micropyle of the fully formed egg during its passage to the outside, and thus the infection is accomplished. (See also Mansour, 1930.) Gier (1936) in working with the "bacteroids" of roaches found that the number of "bacteroids" between the oöcyte membrane and follicle cells increases until there is a uniform layer two or three organisms thick. Before the egg is oviposited, the original oöcyte membrane breaks down and permits the "bacteroids" to enter the cytoplasm. This introduces them into the embryo and a transmission from one generation to the next has been accomplished. No doubt many other new and interesting modes of transmission of the intracellular microorganisms in insects remain to be discovered.

*Nature and rôle of intracellular organisms*

In reading the literature on this subject, one becomes aware of the feeling of uncertainty expressed by various writers as to the true nature of these cellular inclusions. Are they actually living microorganisms? If they are, do they fall into the category of bacteria or of yeasts, or do they belong to that group of microorganisms known as *Rickettsia*?

Some workers (Cuenot, 1896; Henneguy, 1904) have interpreted these intracellular inclusions as mitochondria or as waste

products. The application of various methods of differentiation has shown that mitochondria can usually be distinguished from microorganisms (Cowdry and Olitsky, 1922; Cowdry, 1923). On this basis most workers consider the forms to be living organisms. If they were easily cultivable on artificial media such inclusions would immediately be removed from the category of mitochondria or of waste products. In a large majority of cases, however, very little or no success has been had in the attempts to cultivate these agents artificially.

Two investigators, Pierantoni (1910) and Peklo (see Uichanco, 1924), have reported successful cultivation of the symbionts in aphids. As Uichanco (1924) points out, however, neither of these workers "appears to have furnished adequate evidence to prove the identity of their artificially obtained microorganisms with the 'microorganisms' of the aphid 'mycetocytes'." On the other hand, the symbionts of *Pulvinaria innumerabilis* Rath. have been cultivated by Brues and Glaser (1921). In this case the microorganisms are found in the blood and adipose tissue.

Hertig (1921) was unable to culture the "bacteroids" of the Blattidae and decided that the organism (*Bacillus cuenoti*) cultured earlier by Mercier (1907) from these insects was a contaminant. Glaser (1930a) has reported the cultivation of the microorganisms from the American roach, *Periplaneta americana*. To the diphtheroidal forms isolated he gave the name *Corynebacterium periplanetae*, var. *americana*. In the same year Glaser (1930b) cultivated from the German roach, *Blattella germanica*, an organism he designated as *Corynebacterium blattellae*. On the other hand, Gier (1936) indicates that only negative results were obtained in his attempts to cultivate the "bacteroids" from roaches. In a personal communication, Gier asserts that he made hundreds of attempts to cultivate these forms on many kinds of media, but always without success. He believes that Glaser's diphtheroids are slow-growing contaminants of rather unusual character. He holds the results of Bode (1936) as well as those of Mercier (1907) to be due to poor techniques. Gier was unable to get any signs of growth on chick



chorio-allantoic membranes or in amniotic fluid either with the living chick or in a test tube. Glaser (personal communication) explains the failure of other workers to duplicate his results as due to faulty technique. He maintains that repeated transfers from a medium which has been inoculated with these microorganisms, but which is apparently sterile, are necessary before the growth of the organism finally appears as tiny pin-point colonies. According to Glaser, a period of slow adaptation to artificial conditions apparently is necessary. Glaser also believes that successful cultivation may depend not only on the age of the roach but on the season of the year as well. (See also Neukomm, 1927a, b.)

It is interesting to note that Gier found the "bacteroids" doubling their numbers in the embryos and young nymphs in about ten days. With the rate of increase, the numbers diminished as the animals neared maturity, and apparently decreased in adults except in the ovaries. Long-continued starvation, sublethal doses of X-rays or ultraviolet light, and the injection of crystal violet, hexylresorcinol, and metaphane brought about a decrease in the numbers of microorganisms.

In the writer's opinion, biologists should hesitate to conclude that these forms are not microorganisms simply because of the difficulties encountered in cultivating them. This fault may lie in the inadequacies of the methods and not in the nature of the organisms themselves. The possibility that many of these intracellular microorganisms are closely related to rickettsiae, a group which has not yet been thoroughly defined or given its taxonomic boundaries, should not be overlooked. How close a relation exists between rickettsiae and bacteria has been far from determined. There is already some indication of a taxonomic overlapping of these two groups. In some cases it is fairly certain that the intracellular organisms are of the nature of true bacteria, while in other instances they are definitely rickettsiae. Between the two lies an already large group of non-pathogenic intracellular forms that possess certain characteristics of both. The surveys of Cowdry (1923) and of Hertig and Wolbach (1924)

make the biologist aware of the fact that here lies a large field waiting to be explored, not only from the purely biologic standpoint but for taxonomic reasons as well.

It seems very probable that in the past much of the difficulty encountered in culturing these intracellular organisms was due to the imperfect culture methods used. Many of the early workers went no farther than to use ordinary nutrient agar or broth in their culture experiments. Modern bacteriologic techniques and media have been much improved since that time. The application, in recent years, of tissue culture and chick embryo methods in growing rickettsiae and viruses suggests the possibility of these techniques for the cultivation of the intracellular organisms of insects. Perhaps the difficulty encountered in culturing these forms depends on the extent of their dependence on the host. That is, the closer a microorganism is to being a true and absolute symbiont, the more difficult it may be to cultivate in a foreign environment. Here, indeed, is a branch of the subject that requires expansion.

When we wish to consider the true rôle of these intracellular microorganisms, we find ourselves in a sea of uncertain speculation with only a few small fragments of possible explanations. There is a trend, in much of the literature, toward considering these cellular inhabitants as true symbiotic microorganisms. Others feel that they represent some sort of non-symbiotic parasites. Some consider the bacteria-containing organs, the mycetomes, as analogous to plant galls produced by the host as a response to the irritation by a foreign inhabitant. The microorganisms have also been regarded as true parasites to which the insect has developed an immunity. (See Mansour, 1934a; Paillot, 1931.) Since the physiologic action of these organisms remains so obscure, little is known as to the exact benefit or harm to their host.

It is often quite difficult to make a definite distinction between symbiosis and parasitism. It is generally recognized that there are degrees of symbiosis, that is, symbiosis may be obligate or facultative. In the case of the intracellular organisms in insects, very little work has been done to determine whether or not the

association between these two forms is indispensable to both. It is very probable, however, that both the obligate and the facultative types of symbiosis exist between insects and their symbionts. In any case, it is sometimes difficult to imagine just what constitutes the "mutual advantage" to the two forms of life concerned. Although there are some experimental data on the benefits secured by the host through such a relationship, the evidence for the advantage to the symbionts is considerably more uncertain and hypothetical. This is indicated in a statement by Meyer (1925): "Buchner's suggestion that the intracellular organisms are benefited by being protected within the host from the drastic atmospheric influences of heat, cold, desiccation, etc., is a trifle unreasonable."

The reasons why these organisms have been generally considered to be symbiotic in nature rather than truly parasitic have been set forth by Glaser (1920) as follows:

- (a) Every individual of a species is infected.
- (b) The infection produces changes in the host cells, but these are harmless.
- (c) The infection routes and methods of localization, while different in different hosts and symbionts, follow very definite courses within a species.
- (d) The microorganisms are numerically controlled by the host, never increasing up to a point where they prove fatal.
- (e) The microorganisms within the insects obtain nourishment and protection from drastic temperature and drought conditions.

To these reasons may be added the experimental evidence presented by Aschner (1932, 1934) and Aschner and Ries (1933) who obtained results which, in their opinion, warranted the conclusion that the symbionts play an essential rôle in the life of the body louse. It was found that if the louse *Pediculus* is deprived of its symbionts by operative removal of the mycetome or through elimination of the symbionts by centrifugalisation of the egg, its powers of nutrition and reproduction are greatly impaired. Without the organisms the larvae die and, according to Aschner, it is justifiable to consider this relationship a true symbiosis.

Some workers have thought that in certain insects the close association of the symbionts with the intestinal tract indicates that the microorganisms might aid in digestion (See Buchner, 1930). There is, however, little evidence to support such a view. As stated by Wigglesworth (1939), if these are of value it appears more likely that they contribute to nutrition or metabolism. The process of reproduction in some aphids seems to be intimately associated with the activity of the symbionts. In some cases the latter are thought to synthesize protein for their host during reproductive activity. Wigglesworth also suggests the possibility that they provide accessory factors, and thus enable their host to live permanently on a restricted or highly specialized diet deficient in some respects.

In general, it appears that although in some cases symbionts may not always be essential to life, it is reasonably certain that in other cases they serve a useful purpose. (See also Ries, 1932, 1935; Aschner and Ries, 1933; Koch, 1936a, b.)

Another theory to account for the apparent harmlessness of these intracellular forms is that they have but reached a certain stage in an evolutionary process of adaptation. This, of course, has already been suggested by various writers, especially in the case of the rickettsiae associated with ticks. It is not difficult to imagine that in their initial association, these microorganisms were actually pathogenic parasites, later assuming a more or less commensal relationship until finally a definite mutualistic association was established between the host and the invaders. Certainly the association of these two forms of life must have been an extended one, especially in view of the congenital transmission of the organisms, and for the reason that most insects seem to have such an intimate connection with these forms. It might be emphasized, however, that in certain ants and in the weevil *Calandra granaria* the mycetome is present but apparently contains no symbionts. Some writers have interpreted the formation of mycetomes as the development, on the part of the host, of highly specialized organs whose sole function is that of harboring the symbionts. Such a development is certainly not beyond the range of possibility. Various types of response are known to

result from the entrance of microorganisms into the tissues of plants and animals. As stated by Wallin (1927), these responses, in general, may be both physiologic and morphologic. The presence of mycetomes and bacteriocytes could probably be cited as an example of morphologic response on the part of the host to the intracellular inhabitants. (See also Lilienstern, 1932; Mansour, 1934a.)

Because of the wide disagreement as to the true physiologic nature of these intracellular organisms, Gier (1936) sought to avoid such terms as "symbiont," "symbiosis," "parasite," and "parasitism" and the implications arising from their usage. Accordingly he designated the intracellular bodies in roaches as "bacteroids." No doubt there is considerable justification in using such a term if it is taken to mean "bacteria-like." However, care must be taken in using a designation which has already a definite meaning in soil microbiology. The term "bacteroid" was first used by Brunchort (1885) in referring to the root nodule organisms in the legume plants. As defined by Fred, Baldwin and McCoy (1932), this term is used to designate "the enlarged, frequently club-shaped or branched, vacuolated or banded forms of the root nodule bacteria, both as they occur in the nodule and in culture media." Simply because comparable forms are found associated with insects is no valid reason why these cannot be called "bacteroids." Nevertheless, the same objection may be raised in this instance as that made in the case of the root nodule bacteria by Löhnis and Smith (1916). They objected to the use of the term "bacteroid" because it is now generally recognized that these are forms of true bacteria and not plant products resembling bacteria. Similarly, when the intracellular forms in insects are more thoroughly studied, and perhaps some placed among the bacteria, they then will deserve more than the mere designation of "bacteria-like."

There is considerable danger in too great a reliance upon pathogenic characteristics in determining the taxonomic grouping of microorganisms. Many writers feel that unless an intracellular organism is pathogenic for higher animals it should not be grouped with the rickettsiae even though its morphologic characters are

compatible with such a grouping. Merely because the rickettsia-like organism, such as the one (*Rickettsia lectularia*) in the bedbug (Arkwright et al., 1921; Buchner, 1921c, 1923; Pfeiffer, 1931) is of the symbiotic or commensal rather than of the pathogenic type, such as *R. prowazeki*, is no reason why the non-pathogenic form cannot be legitimately considered a member of the *Rickettsia* group. As pointed out by Hertig and Wolbach (1924), "Bacteria botanically related, for instance the acid-fast bacteria, show comparable extremes in nature of habitat and pathogenicity." The taxonomic characteristics of the rickettsiae as a group are still so imperfectly defined that it seems very likely that its boundaries will be considerably modified before a definite systematic basis is established. There are already those who feel that the term *Rickettsia* should be reserved as a generic name of a particular group of these organisms, such as *R. prowazeki* and those related to it (Macchiavello, 1938). Other types of rickettsiae and rickettsia-like organisms may then be assigned other generic names under the proper family (Rickettsiaceae?). An example of this is the generic name "*Wolbachia*" suggested by Hertig (1936) for the genus of which *Wolbachia pipientis* is the type species.

Kligler and Aschner (1931) give an excellent statement of this phase of the problem: "... further advances in our knowledge of this group of microbes depends on a greater familiarity with the flora of insect parasites in general, and, more particularly, with the group of organisms having the general characteristics of *Rickettsia*. . . . Knowledge of the cultural properties of the non-pathogenic members of this group of microbes may aid in the study of the pathogenic members of this group."

#### OTHER BIOLOGIC RELATIONSHIPS BETWEEN MICROÖRGANISMS AND INSECTS

One cannot hope to include all the biologic relationships between microörganisms and insects within the limits of this review. For this reason, the relatively large field of insect diseases has not been included. It would be inexcusable, however, not to mention this subject as one of the important biologic relationships

existing between these different forms of life. The use of pathogenic agents in controlling insect plagues has been attempted with varying degrees of success. (See Sweetman, 1936.) It is evident that progress in our knowledge of disseminating or controlling the diseases of insects has only begun. This is likewise true of the study of the immunologic aspects of insect diseases. Huff (1940) has included this phase of the subject in a recent review of immunity in invertebrates. Linked with this is the possibility of a better understanding of the principles of epidemiology through the observation of diseases in large insect populations.

Many more examples of the biologic relationships between microorganisms and insects could be added. Pospelov (1926) believes that symbiotic organisms in the fat-cells of certain Lepidoptera serve as food for the developing genital glands and other imaginal tissues. Mahdihassan (1928 to 1935) has been able indirectly to differentiate species of coccids by examining blood smears containing their symbiotic microorganisms which show morphologically distinct forms dependent on the species of insect harboring them. Although according to Snodgrass (1935, p. 50) chitin is not attacked by mammalian digestive enzymes, it is, according to Benecke (1905) broken down by *Bacillus chitinovorius*, which, as stated by Snodgrass, may be the agent of its decomposition in nature. The relation of the phytotoxic secretion of *Pseudococcus brevipes* to its symbiont has been studied by Carter (1935, 1936). Black (1939) found that the juices of insect vectors inhibit the infectivity of tobacco-mosaic virus for Early Golden Cluster Beans. Bacteria have been shown to be able to change the eye color in *Drosophila* flies (Tatum, 1939). The change from white to brown color in the eyes is caused by a "hormone" produced by the microorganisms. These and many more examples indicate the various interesting aspects of this field which could be pursued to good advantage.

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